Mysid *Holmesimysis costata* 7-Day Chronic Toxicity Test

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of water samples, juvenile mysids are exposed to test solutions for 7 days, after which the percentage mortality is determined in each toxicant concentration. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as pH, dissolved oxygen, salinity, temperature, and ammonia.

In this procedure, water samples collected from field stations are divided into replicate beakers in the laboratory. Five randomly selected juvenile mysids are placed into each replicate container. Each beaker is monitored daily for mortality. After a 7-day exposure, survival is counted and recorded to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (US EPA 1995).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (SOP).

2.1 Collection and Culture

- 500-µm-mesh hand nets (~ 25-cm diameter opening)
- 20-liter plastic buckets with tight fitting lids
- airstones and portable aeration (pumps or compressed air or oxygen)
- aerated, flow-through circular culture tanks w/ 150µm mesh screened outflow
- 2-mm-mesh screened brooding compartment to separate juveniles from adults
- nylon screening (100-µm, 150-µm, 500-µm, 2-mm)
- 20-µm-filtered and 1-µm-filtered seawater from a uniform source
- brine shrimp *Artemia* cysts for producing *Artemia* nauplii
- smooth glass tubes; 5-mm-bore, 15-cm length, with suction bulbs (for handling adults)
- wide-bore 10-ml pipette or glass tubes; 3- to 4-mm-bore (for handling juveniles)

2.2 Test Initiation

- 1000 ml clean glass beakers (5 per sample concentration)

• 1000 ml plastic tripour reference toxicant test containers
• 1000 mL volumetric flasks (2) and pipettes for reference toxicant dilutions
• plastic randomization cups (~ 100 ml, one for each test container)
• plastic, screen-bottom tube (150-µm-mesh, 25 cm diameter for mysids)
• plastic, screen-bottom tube 100-µm-mesh for *Artemia*)
• water bath or environmental chamber
• Randomization sheet to arrange and identify test containers
• Data sheets
• Gloves and appropriate safety gear (see MPSL lab safety manual)
• sample vials for reference toxicant analysis (new polyethylene 30 ml, acid washed)
• volumetric pipettes: 1-, 5-, 10-, 25-, and 100-ml
• graduated pipettes: 1- and 10-ml
• volumetric flasks: 1 liter (glass for effluents and organics, plastic for trace metals)
• analytical balance
• plastic squirt bottles

2.4 Water Quality

• Meters and probes for measuring pH, dissolved oxygen, and ammonia
• Refractometer for measuring salinity
• Thermometers (glass mercury thermometer and continuously recording chart thermometer)
• Centrifuge, spectrophotometer and reagents for measuring sulfide (see sulfide SOP)
• Graduated pipettes (10 ml) and hand pipette pump for water quality sampling
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

Dilution water consists of ambient Granite Canyon seawater, filtered to 1 µm, at ambient salinity (33-34‰).

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of five replicate test beakers for each sample concentration. Beakers are arranged randomly, and each receives five randomly selected mysid juveniles. The quality of test

mysids and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and pH, NH_3, salinity, ammonia, and dissolved oxygen are measured at the beginning and end of the exposure. Temperature is measured continuously.

4.0 SAMPLE PREPARATION

Label one-liter beakers as indicated on the randomization sheet generated for the test. Determine the salinity of the test solutions. Be sure to stir all samples before measuring salinity, and measure salinity immediately after stirring. Samples salinities below 32‰ are adjusted to 34‰ using hypersaline brine made from frozen seawater or artificial salts. Check the pH of brine. If necessary, adjust the brine pH by adding acid or sodium hydroxide until it is between 7.5-8.5.

Using the random number sheet, aliquot 200 mL of sample to beakers. Minimize sample exposure to sunlight (never leave samples in direct sunlight), and schedule loading times to avoid prolonged sample exposure to temperatures above 15°C.

5.0 CONTROLS

5.1 Seawater and Brine Controls

The seawater control consists of 20 µm filtered Granite Canyon seawater. If a brine control is necessary, it should be prepared to contain the same proportion of brine as the lowest salinity sample that was adjusted.

5.2 Reference Toxicant Tests

A reference toxicant test must be conducted concurrently with every test to indicate the sensitivity of the organisms and the suitability of the test methodology. Zinc sulfate ($ZnSO_4$) should be used as the reference toxicant for mysid tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 10,000 µg/liter zinc stock solution by adding 0.0440g $ZnSO_4$ to one liter of distilled water in a volumetric flask. Cap tightly and mix thoroughly. Sample the reference toxicant stock solution at the beginning of the test for chemical verification of the zinc concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N reagent grade nitric acid. Reference toxicant solutions consist of five replicates of the following concentrations: 0, 10, 18, 32, 56, and 100 mg/liter. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare 200 ml of each concentration.

All tests (sample and reference toxicant) must use juveniles from the same pool of gravid females. They must be handled in the same way and delivered to the test containers at the same time.

6.0 TEST ORGANISMS

6.1 Collecting and Isolating Mysids

The mysid shrimp, *Holmesimysis costata*, commonly inhabits the surface canopy of the giant kelp *Macrocystis pyrifera* where it feeds on zooplankters, kelp, epiphytes, and detritus. Mysid sensitivity to contaminants, their ease of laboratory culture, and their ecological importance make them suitable organisms for determining the toxicity of chemical compounds and complex effluents. Collect mysids about 7 days prior to test initiation using 500 µm mesh dip nets. To isolate juveniles, place females carrying embryos in the eye-development stage in brood compartments within holding tanks. Juvenile mysids released over a twenty-four hour period are isolated and transferred to a separate tank until enough are collected for test initiation. Three to four day-old juveniles are used in the test.

6.2 Randomized Loading of Mysids

Transfer juvenile mysids from their holding aquarium into a shallow container or screen tube for sorting. Using a 5-mm bore pipette, transfer one to two mysids at a time into test containers. Repeat this process until all beakers are loaded. Place the acrylic covers over each set of beakers on a shelf. Maintain water temperature (15°C ± 1) by sorting animals in the constant temperature room where the test is being held.

7.0 MONITORING THE TOXICITY TEST

7.1 Counting Mysid Mortality and Feeding

Test duration is 7 days. Check all test containers daily, and record the number of dead mysids. Immobile mysids that do not respond to a stimulus are considered dead. The stimulus should be two or three gentle prods with a disposable pipette. Mysids that exhibit any response clearly visible to the naked eye are considered living. Remove dead animals, *Artemia* and other debris. Mysids are fed 20 freshly hatched *Artemia* nauplii per mysid twice daily.

7.2 Measuring Water Quality in Test Containers

Measure temperature, dissolved oxygen, pH, and ammonia in the water of one replicate from each sample at the beginning and end of the test. Sample the initial test solutions at the time of dilution preparation. Water quality should also be measured on old and new dilutions at the time of renewal. Renewal water quality parameters include: dissolved oxygen, pH, and temperature.

8.0 TEST SOLUTION RENEWAL

Because toxicity may change over short time periods in test containers, the test solutions must be renewed after 48 and 96 hours. Dissolved oxygen concentrations should be checked on new water used and these samples must be aerated if DO concentrations exceed maximum values allowed.

Remove 50% of the original test solution from each container, taking care to avoid losing or damaging mysids. This can be accomplished by siphoning with a small-bore (2 to 3 mm) fire-polished glass tube or pipette. Attach the glass tube to clear plastic tubing fitted with a pinch clamp so that the siphon flow can be stopped quickly if necessary to release entrained mysids. Follow the container randomization sheet to siphon first from the controls, then work sequentially to the highest test concentration to avoid cross-contamination. Siphon tubes must be leached for 24 hours prior to renewal in seawater.

To minimize disturbance to the juvenile mysids, refill the containers to the 200 ml mark by carefully siphoning new test solution into the test containers using small diameter plastic tubing attached to a bent clean glass rod that directs incoming upward or to the side to slow the current and minimize turbulence. Feed 20 *Artemia* nauplii per mysid to each test container after the renewal solution has been added.

9.0 TERMINATING THE TOXICITY TEST

After 7 days of exposure final mortality counts are made. Final water quality must be sampled at the termination of the test. Deliver a sample from each site into pre-labeled water quality containers. Measure D.O., pH, ammonia, and temperature of each sample.

10.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and brine controls have acceptable survival (mean of ≥ 75%). If not, notify the project officer without delay. This toxicity test procedure is considered acceptable if mysid survival in dilution controls is greater than or equal to 75%. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from 13 ± 1˚C; acceptable dissolved oxygen concentration is 60-100% saturation.

11.0 REFERENCES

U.S. Environmental Protection Agency. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. Office of Research and Development. EPA/600/R-95/136. August 1995

12.0 TEST SUMMARY

Species:	*Holmesimysis costata*
Test Duration:	7 Days
Renewals:	One at 48 hours
Organism Source	Wild-caught adults from local kelp beds
Age of test organisms:	3 to 4 days post-hatch
Test Salinity:	Ambient ± 2‰
Test Temperature:	15 ± 2°C
Light intensity:	Ambient laboratory illumination 10-20 µE/μ^2/s
Photoperiod:	16 hour Light : 8 hour Dark
Replication:	5 replicates
Test Containers:	1000 mL beakers
Test solution volume:	200 ml minimum
Loading:	5 animals per beaker
Feeding:	Feed 20 newly hatched *Artemia* nauplii per larvae twice daily (morning and night)
Water Quality:	pH, D.O. temp, and NH_3
Reference Toxicant:	Zinc sulfate ($ZnSO_4$)
Stock Solution:	0.0440 g in 1 liter of dist water
Dilutions:	0, 10, 18, 32, 56, and 100 mg/L (0, 1, 1.8, 3.2, 5.6, and 10 ml stock/L)
Daily Monitoring:	Count number alive and remove dead
Safety:	Wear protective clothing; read applicable MSDS, be familiar with the lab safety manual prior to testing.
Quality Control:	Fill out all data sheets completely. Be familiar with QA Project Plan prior to testing.
Acceptability Criteria:	Seawater and Brine Controls: >80% Temperature range: 13° to 17°C. Dissolved oxygen range 4.91 to 8.19 mg/l